



# ANIMAL TRACKS

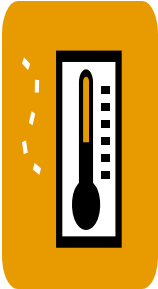


A newsletter for the Duke research community

July 2007

<http://www.duhs.duke.edu>

## Important Points On Anesthesia: Monitoring Anesthesia



All anesthetized animals must be monitored continually to assess adequate level of anesthesia. Monitoring must begin with the first injection (or inhalation) of anesthesia and continue until the animal is fully recovered (or euthanized). There are as many acceptable methods to monitor anesthesia as there are species of animals. Certain methods, commonly used in most species, These include:

- **Toe pinch:** A gentle pinch, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal requires more anesthesia. Any observed movement (e.g. withdrawing the paw) indicates that the animal is not sufficiently anesthetized to do surgery, or any painful procedure.
- **Skin pinch:** More sensitive areas of skin work best. A gentle pinch of a small fold of skin, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal requires more anesthesia, Any observed movement (e.g. twitching of the skin) indicates that the animal is not sufficiently anesthetized to do procedures.
- **Jaw "tone":** Generally a good indicator of muscle relaxation. The lower jaw is gently opened. Resistance by the animal (e.g. closing of the mouth) is an indicator that the animal is too light to do surgery.
- **Respiratory rate:** Good indicator of depth of anesthesia. Rapid, shallow respirations usually indicate the animal is too "light". Normal respiration rate varies among species, consult DLAR veterinarians if necessary for determination of normal rates.
- **Heart rate/Blood pressure:** An increase in heart rate and/or blood pressure usually indicates a need for supplemental anesthesia. Normal rates/pressures vary greatly among species, consult DLAR veterinarians if necessary for determination of normal rates/pressures.
- **Palpebral:** While useful in large species. the blink reflex is quite variable and difficult to assess in small animals (i.e. mice and rats). **DO NOT USE FOR MONITORING RODENT ANESTHESIA!**



## Division of Laboratory Animal Resources Veterinary Diagnostic Laboratory (VDL)

The Division of Laboratory Animal Resources provides diagnostics for research animals within the Duke program for animal care & use. Services routinely available to Duke researchers include:

- ◆ Complete Blood Counts with automated five part differential (Cell Dyn 3700 Analyzer)
- ◆ Cell Line Testing for Murine Pathogens
- ◆ Hematology
- ◆ Serology
- ◆ Virology
- ◆ Chemistry
- ◆ Molecular Diagnostics
- ◆ Q-Fever Immunofluorescent Assay
- ◆ Parasitology
- ◆ Urinalysis
- ◆ Cytology
- ◆ Microbiology
- ◆ Sentinel Program
- ◆ Environmental Monitoring/water quality testing
- ◆ Necropsy / Histology / Pathology
- ◆ Import/Export Program



To arrange for any of these services, please see the contact list on Page 3!

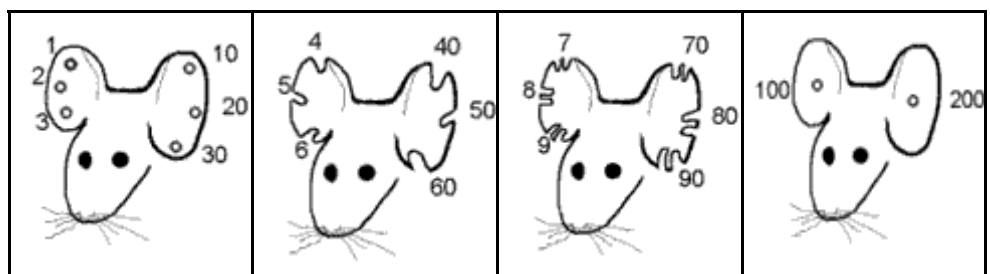
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## RODENT IDENTIFICATION

Individual identification of animals plays a critical role in accurate record keeping. There are several methods of identification of rodents. Proper restraint plays an important role in most of these techniques. **All these techniques are acceptable forms of identification, but MUST BE APPROVED AS PART OF YOUR DUKE ANIMAL PROTOCOL.**

- **Metal ear tags** are inexpensive and don't require anesthesia for application (although this may facilitate proper placement) but the animal must be securely restrained. The tags need to be appropriate size for the species and should be applied to the distal 1/3 of the pinnae. If placed correctly they generally last for at least 6 months. There is a possibility of local infection and implantation sites should be monitored occasionally.
- **Tail Tattoo** is an acceptable technique and offers a permanent means of identification. Disadvantages include initial cost of equipment, some skill and experience is needed and the need for anesthesia. Good for long term studies, especially in rats.
- **Electronic Transponders** are a recent option. Anesthesia is not required, but may facilitate placement, as this only requires a subcutaneous injection. Initial cost for reader is high but this is useful method for accurate identification. Toe clipping is a traditional method of identification and has the added benefit of high quality tissue sample for analysis. However, the IACUC discourages toe clipping because it has the potential to cause pain and distress and might alter the gait, weight-bearing ability of a limb, and ability to feed.
- **Toe clipping** can be considered under the following conditions: 1) Alternative methods of identification must first be considered. 2) A written explanation of why it is necessary is required, including a discussion of why alternate methods are unsatisfactory. This will be considered and must be approved by the IACUC. 3) It should only be performed when mice are between 5-10 days of age and is limited to only one digit per extremity. Mice should be anesthetized. Sharp sterile scissors should be used and the foot should be cleaned with a dilute betadine solution.
- **Ear notch / ear punch** is another identification option. It is inexpensive and permanent, the animal must be securely restrained but no anesthesia is required if the animal is less than 3 weeks of age. Ear clipping remnants can usually provide enough DNA for an initial PCR screening. A commonly accepted ear notch punch code system is:



- **Corneal:** The cornea can be damaged, if not protected, but when used carefully, it is a good reflex. Touch the edge of the cornea with a gauge sponge or cotton q-tip. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.
- **Body Temperature:** Most anesthetic agents depress body temperature to a significant degree. Therefore, it is important that anesthetized animals be maintained on some type of material which shields them from contact with cold surfaces and reduces heat loss. The use of a supplemental heat source is a good idea, but must be used with caution, since burns can occur from electric blankets or water bottles that are too hot.

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### Anesthesia Recovery

All animals recovering from anesthesia must be attended until they have recovered their swallowing reflexes. In most species, this is usually indicated by the animal moving purposely. All animals should be kept warm during the recovery period. While heat lamps have been used, there is a significant risk of heat injury if not properly attended. If using a heating pad, place the animals in a cage that is 1/2 on and 1/2 off of a heating pad. Whenever a heat source is used, a thermometer should be placed at the animal's level to monitor actual heat levels. Animals will overheat and can 'sun burn.' Animals which have had any blood/fluid loss during surgery should be provided with fluid or blood replacement during surgery and/or the anesthesia recovery period. In small rodents, this is best accomplished via the intraperitoneal or subcutaneous route. A warmed cc or 2 of sterile saline given SQ is always a good idea in small mammals.

### Contacts for DLAR's VDL:

- Dr. Yohannes Asfaw, DVM**, Head of VDL: 684-3802, [Yohannes.asfaw@duke.edu](mailto:Yohannes.asfaw@duke.edu)  
**Kristy Jackson**, VDL Manager: 684-4512, [Kristy.jackson@duke.edu](mailto:Kristy.jackson@duke.edu)  
**Miriam Coltrane**, VDL Technician II: 684-0821, [Miriam.coltrane@duke.edu](mailto:Miriam.coltrane@duke.edu)  
**Susan Glowacz**, VDL Technican II: 684-0823, [Susan.glowacz@duke.edu](mailto:Susan.glowacz@duke.edu)  
**Torie Anderson**, Import/Export Coordinator: 684-2478, [Torie.anderson@duke.edu](mailto:Torie.anderson@duke.edu)

### Upcoming Dates & Deadlines

July 5	Significant Change Meeting
July 9	New Protocol & SC deadline
July 19	Significant Change Meeting
July 23	SC deadline
July 26	IACUC meeting
August 2	SC deadline
August 6	New Protocol & SC deadline

Deadlines are 5 PM on the date listed! No exceptions!

## Duke University Medical Center DLAR Veterinary Diagnostic Laboratory (VDL) Vivarium Building Room 0001

### *Sample submission requirements*

#### Blood for Hematology

- Draw whole blood into a lavender or EDTA tube (50-80% full).
- Immediately mix thoroughly by inverting several times to avoid clots.
- Refrigerate at 4°C and submit blood sample within 8 hrs to VDL.
- Contact lab for information to submit rodent blood samples.

#### Serum for routine clinical chemistry

- Collect blood in a tube with serum separator gel or plain red top tube.
- Mix the tube by 5 complete inversions.
- Allow blood to clot at room temperature for a minimum of 30 minutes then centrifuge.
- Centrifuge at 2000-3500 rpm for 10-20 minutes as soon as possible. ([www.bd.com/vacutainer](http://www.bd.com/vacutainer) for more details).
- Submit serum sample either in the same tube or after transferring to a transport tube.
- Store sample at 4°C and submit sample to VDL as soon as possible.

#### Swab for bacteriology

- Swab specimen in culturette- type transport semi-solid media (aerobic or anaerobic).
- Seal caps with paraffin or tape to prevent leakage.
- Activate ampule-type culturettes immediately.
- Submit sample to VDL as soon as possible.

#### Fecal sample for parasite check (Direct and Float)

- Place fecal sample in fecalyzer.
- Store samples at 4°C and submit to VDL as soon as possible.

#### Cell line testing

- Samples sent to MU RADIL for murine pathogen screening.
- Contact Kristy Jackson or lab staff for submission requirements.

Samples should be submitted to Veterinary Diagnostic Lab between the hours of 8:00 am-3:00 pm Monday to Friday. Certain tests and samples are time sensitive and restricted by shipping deadlines. Prior arrangement is required for large number of samples. Blood tubes and other specimen collection materials can be obtained from VDL.

Please contact Kristy Jackson (684-4512), Miriam Coltrane (684-0821), Susan Glowacz (684-0823) or Dr. Yohannes Asfaw (684-3802) for additional information.

# Recognition and Treatment of Pain

In the Animal Welfare Act, a painful procedure is defined as, "... any procedure that would reasonably be expected to cause more than slight and momentary pain or distress in a human being..."

In both humans and most animals, the total pain experience results from an interaction between sensory pathways and the affective system, which provides the motivational and emotional component of pain. This varies considerably between species and individuals within a species.

Understanding the degree of pain involved in various experimental procedures provides an avenue of prediction of animal pain or distress. Physiological responses to pain can include increased blood pressure and heart rate, pupillary dilation, increased respiration, and an arousal response on the electroencephalogram. If baseline values are known for these variables, they can be monitored for changes.

To detect behavioral signs of pain, one must be familiar with the animal's normal behavior. It is worth a reminder that: *behavioral responses to pain vary between species, within species, and even within the same animal over different time points.*

General behaviors that could be considered include: sleeping, feeding, drinking, locomotion, grooming, exploration, performance in learning and discrimination tasks, mating behavior, social interactions, and dominance/subservience responses within the social system.

## Typical behavioral signs of acute pain:

- Protecting the painful area,
- Vocalizing (especially when handled or moving),
- Licking, biting, scratching, or shaking the painful area,
- Restlessness,
- Lack of mobility,
- Failure to groom
- Abnormal postures, or
- Lack of normal interest in surroundings.

**Anthropomorphic Reflection of Potential Pain in Animals: Unless there is evidence to the contrary, assume that a procedure that causes pain in humans will cause pain in animals.**

## Other points to remember are:

- Abdominal surgery appears to be less painful in animals than humans, probably because most animals do not use their abdominal muscles for postural support (humans do use abdominal muscles for postural support). However, appearances can be deceiving. Large animal abdominal surgery is more painful than small animal abdominal surgery, probably because of the increased abdominal contents weight of larger species.
- Lumbar and thoracic spinal surgery in animals also appears to be less painful than in man, probably due to man's postural requirements. However, procedures involving the cervical spine appear to be more uncomfortable in animals, than in humans.
- In animals, thoracic surgery involving the sternum appears to be more painful than surgery using a lateral intercostal approach.

- Surgery on the eye, ear or surrounding structures seems to elicit more signs of distress in animals than would be expected by comparing with human surgeries. Signs such as head-tilt or shaking, or pawing or rubbing the area may be seen in animals, when the similar procedure in humans would not cause significant distress..
- Peri-rectal procedures seem to produce significant discomfort in animals (compared to humans). In addition to analgesia, protection of the affected areas is indicated in animals to prevent self or cage mate trauma.
- Surgery of the femur or humerus appears to be painful to most animals, and more painful than human experience, which may be due to trauma to the major muscle groups which overly these two bones.
- Animals in pain tend to distrust new taste or flavors, and if the association between a painful event and a new taste is made, the animal may refuse to consume it. This is one reason why the use of analgesics administered AFTER the painful event had begun is often not as successful as analgesic administration BEFORE the development of pain (the phenomena of neophobia).

## Influences of pain perception:

- Pain perception can be influenced by drugs, environmental, and behavioral factors. **Practical application:** Always place analgesics on board before the painful event.
- Recovery in familiar surroundings may help to relieve distress and some pain. **Practical application:** Whenever possible, let the animal recover in its home cage.
- Acclimatization prior to a procedure may also facilitate recovery. **Practical application:** If you'll be giving analgesics in the water, provide the treated water 3-5 days BEFORE the painful event to acclimate the animal to the taste of the medicated water. **Practical application:** If you are using testing chambers or restraining devices, place the animal in the device daily for 3-5 days BEFORE the test event and do nothing, to familiarize them to the chamber or device.
- The use of pre-emptive analgesia is a highly important concept to consider and employ! **Practical application:** Giving analgesic before anesthesia will decrease the amount of analgesic required, smooth the recovery, and decrease the amount of analgesic necessary post-procedure to control pain.
- The environment should be kept stable, minimizing stimuli that evoke a fearful response in the animal. Always handle the animal in an appropriate manner. **Practical application:** Kind and compassionate handling of the test animal will result in a calmer animal, less likely to bite or fight, and will provide more stable research data!

**Various analgesics are available to the investigator to assist with pain management.** When designing an analgesic section of the protocol, the investigator should consult Duke veterinarians who are experienced in animal pain management and elimination. Interaction of the analgesic with concurrently used drugs and the effect of the agent on study results must be considered when choosing the best agent for a given situation.

## NIH Clarifies Grant Money Policy

The National Institutes of Health (NIH) has issued a notice<sup>1</sup> explaining that NIH grant monies cannot be used for work with live vertebrate animals unless that work is associated with a valid Animal Welfare assurance from the Office of Laboratory Animal Welfare (OLAW) and/or a valid IACUC-approved protocol. The Office of Management and Budget Cost Principles and the NIH Grants Policy Statement (NIHGPS) do not allow for the animal work to be charged against NIH grants in cases when either or both of these conditions are not met.

The NIH notice listed the following as specific situations under which charges are not allowed:

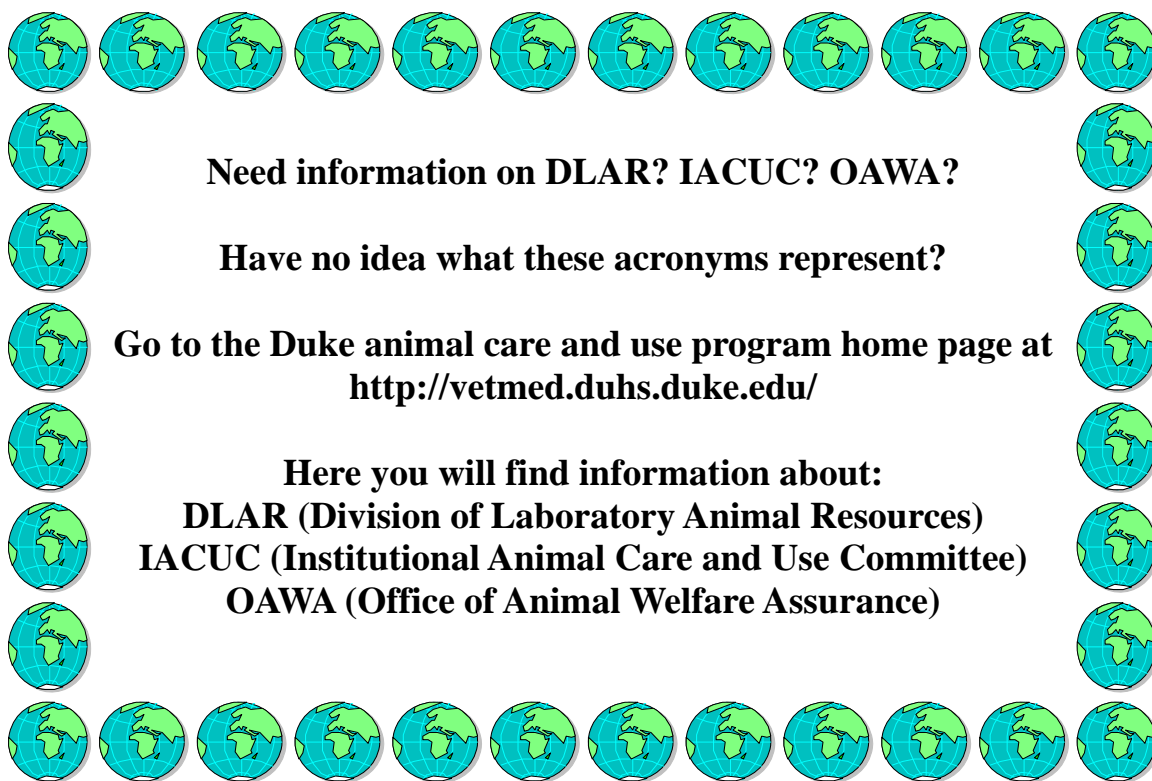
- ⇒ The conduct of animal activities in the absence of a valid Assurance on file with OLAW.
- ⇒ The conduct of animal activities in the absence of a valid IACUC approval of the activity. Absence of IACUC approval includes failure to obtain IACUC approval, expiration, or suspension of IACUC approval<sup>1</sup>.”

Any situations that do not meet these terms and conditions as defined in the NIHGPS should be reported to the Institute or Center supporting the grant. During periods when there is no valid Assurance of IACUC approval, grantees must continue to care for their animals, although whether grant money will be available to go toward such animal care activities will only be decided on a case-by-case basis.

The notice specifies that the above-mentioned situations constitute serious noncompliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals<sup>2</sup> that must be promptly reported to OLAW.

In addition, the notice includes a reminder that the primary grantee is responsible for the conduct of a project involving institutional collaboration, including grant expenditures by all parties. As such, the primary grantee must ensure that collaborating organizations have valid Assurances and IACUC approvals for the animal work they are conducting.

1. NIH. NOT-OD-07-044. NIH Policy on Allowable Costs for Grant Activities Involving Animals when Terms and Conditions are not Upheld. 26 January 2007). <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-07-044.html>.
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* IV.F.3 (US Department of Health and Human Services, Washington, DC, 1986; reprinted 2002).



**Need information on DLAR? IACUC? OAWA?**

**Have no idea what these acronyms represent?**

**Go to the Duke animal care and use program home page at**  
**<http://vetmed.duhs.duke.edu/>**

**Here you will find information about:**  
**DLAR (Division of Laboratory Animal Resources)**  
**IACUC (Institutional Animal Care and Use Committee)**  
**OAWA (Office of Animal Welfare Assurance)**

## OAWA's Brown Bag Seminar

Friday, July 20<sup>th</sup>, 2007

Noon – 1 p.m.

Bryan Research Building: Room 103



**Dr. Ron Banks**  
**Director of the Office of Animal Welfare**  
**Assurance will be presenting:**

## The New Duke IACUC Protocol Template: The “How To’s” for the new system

The Duke IACUC has been working for over a year to develop a new animal use protocol template. The new template has several goals:

1. To develop a ‘back-bone’ document for the soon-to-be-coming web-submission for animal use applications;
2. To have a protocol that asks the questions necessary for the IACUC to make an informed decision;
3. To have a protocol that is easier for researchers to follow;
4. To decrease the number of clarification questions required; and
5. To allow reviewers to be able to review efficiently and completely.

At this seminar, the Office of Animal Welfare Assurance will give a preview of the animal use protocol template and discuss how to use it. The protocol template has a core document that will be completed with each new protocol submission, and then additional sections will be completed and submitted as they apply to the proposed research plan and procedures. We will discuss:

1. General overview of the purpose of a protocol template;
2. General overview of the Duke IACUC’s actions in developing the new template; and
3. General overview of the structure of the new template and the concept of how it will work, by Part and Section.

Hard copies of the template core will be available at the seminar with a web-link for the various appendices for specific sections of the protocol. Dr. Banks will briefly touch on each of the appendices of the template. P.I.s, laboratory managers, and research associates are strongly encouraged to attend to minimize speed bumps during the transition to the new template.

The presentation will be on **Friday July 20<sup>th</sup>, 2007** in room  
**103 of the Bryan Research Building**,  
located at 421 Research Drive, on Duke University’s West Campus.

Attendees are encouraged to bring a lunch. OAWA will provide drinks and desserts. The session will begin promptly at noon. Please arrive early to sign-in and find a seat.

For those who will be coming from off campus, driving directions and parking information can be found at the following link: <http://neuro.duke.edu/Links/map.htm>